ENGLISH LANGUAGE TRANSLATION

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USE OF BETA-NAPHTHOQUINONE DERIVATIVES FOR MAKING DRUGS WHICH EXERT AN INHIBITORY EFFECT ON THE RELEASE OF GLUTAMATE BY THE BRAIN

Novel use of beta-naphthoquinone derivatives and salts thereof for making drugs exerting an inhibitory effect on the release of glutamate in the brain.

The invention relates to a novel use of beta-naphthoquinone derivatives and salts thereof for making drugs exerting an inhibitory effect on the release of glutamate in the brain.

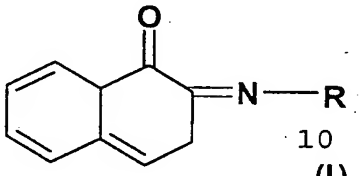
Glutamate is the main neurotransmitter in the central nervous system of mammals.

However, its build-up, in excess, in the extracellular space of the brain is toxic for neurons, which has led to the consideration that it was a causal factor of a large number of neurological diseases, like epilepsy, amyotrophic lateral sclerosis, spinal amyotrophy, Huntington's disease and in different attacks related to deleterious effects due to excesses of glutamate released as a result of cerebral accidents of a traumatic vascular or other origin.

The investigation by the inventors of certain beta-naphthoquinone compounds known as vasoprotective drugs

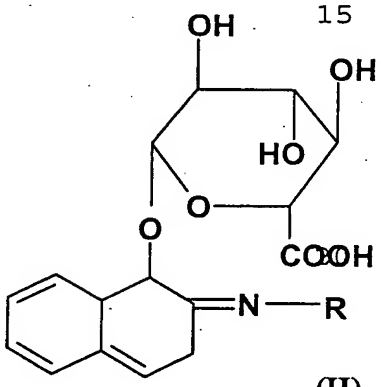
has shown that they unexpectedly have inhibitory effects on the release of glutamate.

The invention is therefore directed to a novel use of beta-naphthoquinone derivatives for making drugs with an inhibitory effect on the release of glutamate by the brain, these derivatives having formula (I)



(I)

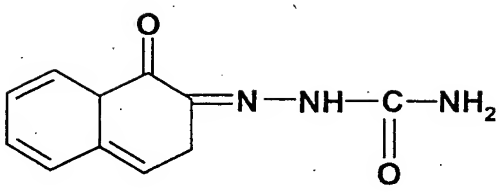
wherein R represents a -NH-CO-NH_2, -NH-CO-CH_3, or -OH group, corresponding glucuronidated derivatives having formula (II)



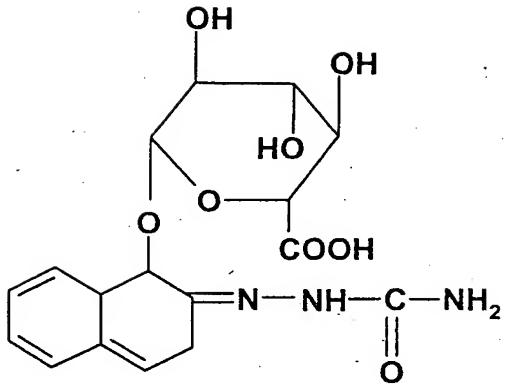
(II)

as well as addition salts thereof with pharmaceutically acceptable acids.

It is most particularly directed to the use of the 1,2-naphthoquinone 2-semicarbazone, called naftazone according to its international common name, as well as to its corresponding glucuronidated derivative, 1-(1-hydroxy,2-naphthyl)semicarbazide-1-β-O-gluco-pyranosiduronic acid, respectively of formulae, (III) and (IV)



(III)



(IV)

The addition salts with acids of these compounds comprise salts formed with mineral acids or organic acids.

As an example, hydrochloric, hydrobromic, sulfuric, phosphoric, or even formic, benzoic, maleic, tartaric, citric, oxalic, aspartic acid, and alkane-sulfonic acids will be mentioned.

The preparation of compounds used according to the invention has been widely described in the literature, for example, in BSM 924 M or Patent FR 2103 504.

The inhibitory properties of these compounds, reported in the examples hereafter, make them particularly suitable for treating neurological diseases and attacks related to deleterious effects of glutamate released in excess.

For example, the treatment of epilepsy, of amyothrophic lateral sclerosis, spinal amyothrophy, Huntington's disease, deleterious effect due to excesses to glutamate released as a result of cerebral accidents of traumatic or other vascular origin will be mentioned.

These drugs are notably administered orally and via an injectable channel. Advantageously, they are in the

form of tablets, sugar coated tablets, hard gelatin capsules, capsules, granules, for oral administration, or solutions or suspensions for administration via an injectable channel.

The doses will be adapted according to the patient and the pathology to be treated and are for example 1 mg-100 mg/day.

Other features and advantages of the invention are given in the following examples, wherein reference is made to Figs. 1 and 2, wherein respectively:

- Fig. 1 represents the diagram illustrating the chemiluminescence measurement protocol, and
- Fig. 2 represents the spontaneous release of glutamate and the induced one versus the concentration of naftazone (Fig. 2A) or of its glucuronidated derivative (Fig. 2B).

Example 1: study of the inhibitory effects on glutamate by naftazone and its glucuronidated derivative.

A: Study of the effect of a continuous treatment with naftazone for 15 days on the glutamate levels in the CSF (cerebrospinal fluid) of normal rats

Sprague-Dawley rats weighing 200-220 g and Swiss-Webster mice of both sexes, aged 4-8 weeks, are used.

The animals are kept in cages, in a well-ventilated room at 23-24°C, with a light/darkness cycle of 12 hours.

In order to investigate the CSF glutamate levels in the controls, or after treatment with naftazone, the male rats are divided into 3 groups:

- Group I (n=8) is used as control. The rats of this group are fed *per os* for 15 days with the same carrier as the one used for solubilizing naftazone,

i.e. 1% methylcellulose (Sigma),

- The animals of groups II (n=5) and III (n=5) are fed *per os* for 15 days with 10 and 100 mg of naftazone per kg, per day, respectively, given as a single bolus.

The CSF of anaesthetized rats with 6% pentobarbital (i.p.) is collected by operating according to the usual procedures.

The animals are then decapitated. The CSF samples are centrifuged at 6,000 g for 10 min at 10°C.

The supernatant is extracted, the sediment containing the blood deposits is removed.

The samples are held in 2.5% trichloracetic acid and kept at -80°C.

Ether is used for washing trichloracetic acid off the samples.

In order to determine the glutamate levels in the CSF, chemiluminescence measurements are conducted according to the procedure described in the diagram given in Fig. 1. The reaction is based on the oxidization of glutamate into 2-oxoglutarate under the action of glutamate dehydrogenase, which produces NaDH2, evaluated by using the chemiluminescent reaction of *photobacterium*.

The CSF samples are tested by adding a known volume of sample to the reaction medium which contains 250 µl of saccharose (120 mM) in Tris buffer (120 mM, pH 7.2), 50 µl of an enzymatic mixture of NAD, DMN, NADH-FMN oxidoreductase, luciferase and GDH, and 5 µl of n-decyl aldehyde.

The light emitted by the luminescent reaction consecutive to the oxidization of L-glutamate and to the production of NADH, is detected by a

photomultiplier unit, recorded and calibrated by comparing it with light emitted by a glutamate standard.

Statistical analysis of the data is carried out by using Student's t test for unpaired samples. The values are expressed as average +/- SEM, n = number of animals or experiments carried out.

The data are considered as significantly different from the controls, at $p<0.05$.

The control rats (Group I) which have received the methylcellulose carrier for 15 days, have a CSF glutamate content from 16-34 nmol ml^{-1} with an average value of 22.1+/- 6.3 nmol ml^{-1} (n=8).

The daily treatment of rats (groups II and III) for 15 days with a naftazone dose of 10 or 100 mg/kg show that the CSF glutamate content in both groups of rats is 8.1 +/- 1.8 (n=5) and 10.8 +/- 3.3 ml^{-1} (n=5), respectively.

These results show that the glutamate content in the CSF of rats treated with both naftazone doses is significantly reduced ($p=0.001$ and $p=0.004$, respectively), as compared with the controls.

Furthermore, no significant difference in CSF glutamate content is observed between both groups of rats treated with naftazone, which shows that the effect of the drug is not dose-dependent.

B: Study of the effect of naftazone and of its glucuronide derivatives on the release of glutamate from synaptosomes of mouse brains.

In order to prepare the synaptosomes of mossy fibers, the Swiss-Webster rats are decapitated and the cerebellum is rapidly removed. Small pieces of tissue

(1-2 mm^3) are washed in 100 ml of a mammal saline standard solution containing (mM): NaCl, 136; KCl, 5.6; $MgCl_2$ 1.2; $CaCl_2$ 2.2; glucose 5.5; $NaHCO_3$ 7.5; $NaHPO_4/Na_2HPO_4$ buffer 1.2.

An oxygen current is caused to flow through them for 10 minutes.

In order to dissociate the pieces, they are sucked in a reciprocal movement with a 1 ml pipette.

The homogenate obtained is diluted in 3 ml of mammal Krebs's solution and is filtered through a Nylon® tissue (mesh 50 μm).

The filtrate is collected and left to settle for 30-45 min by gravity.

Synaptosomes derived from the glutamatergic mossy fibers settle because of their large size with the nuclear fraction. The supernatant is discarded and the sediment is resuspended in 1 ml of a standard solution. The release of glutamate from the synaptosomes is detected according to the technique used for evaluating it in the CSF.

Figs. 2A and 2B show the effects of naftazone (at concentrations of 0.5-50 μM) and of its glucuronidated derivative, respectively, on the spontaneous release of glutamate (curve -o-) and of that induced by depolarization (curve -•-).

Each point in A and B represents the ISEM average of 3 measurements carried out in triplicate. In A, the spontaneously released glutamate is continuously measured during an exposure of 1 hour to the tested drug and is compared with controls. Release of glutamate by depolarization is determined after a 1 hour exposure to the tested drug and is compared with controls.

The drugs are left to incubate for 1 hour with synaptosomal aliquots before the measurement.

The release of glutamate in response to the depolarization induced by a medium with a high K^+ content (30 mM) containing Ca^{2+} (5 mM) is not significantly affected by naftazone at the investigated concentration values.

However, as Fig. 2A shows, naftazone reduces the spontaneous release of glutamate from synaptosomes. The inhibitory effect of naftazone on the spontaneous release of glutamate is already observed at the lowest concentration of drug used (0.5 μM). This effect is maximal at the concentration of 25 μM.

Higher concentrations do not seem to further increase the inhibitory effect.

When the effect of the glucuronidated derivative on the spontaneous release and on that caused by K^+ is evaluated, it is seen that the drug does not reduce the spontaneous release of glutamate in the range of the concentrations used.

However, as Fig. 2B shows, the glucuronidated derivative reduces, in a dose-dependent way, the release induced by a medium with a high K^+ content (20 mM) containing Ca^{2+} (5 mM).

The maximum reduction (about 60%) is observed at the highest concentration of the tested drug (32 μM).

Example 2: Preparation of pharmaceutical compositions.

By operating according to the conventional techniques, tablets are made containing:

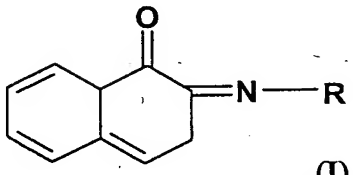
- naftazone: 10 mg
- excipient qsp for 100 mg

or injectable solutes containing:

- naftazone: 5 mg
- sterile water qsp: 2 ml.

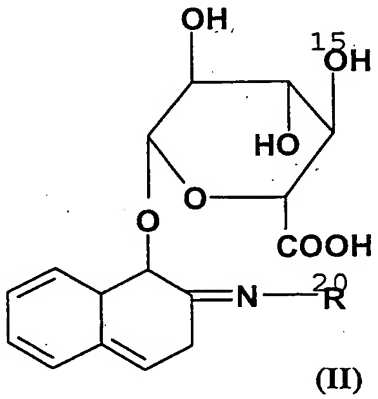
CLAIMS

1. The use of beta-naphthoquinone compounds for making drugs having an inhibitory effect on the release of glutamate on the brain, said compounds having the formula (I)



(I)

wherein R represents a -NH-CO-NH_2, -NH-CO-CH_3, or -OH group, corresponding glucuronidated derivatives of formula (II)

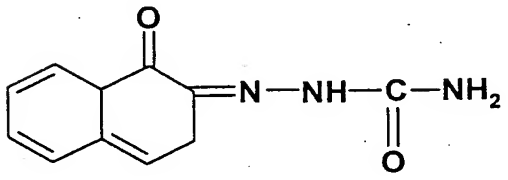


(II)

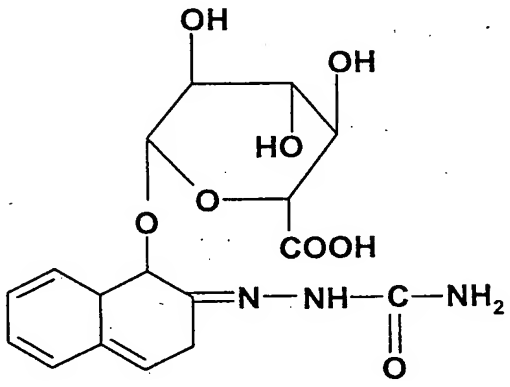
as well as their addition salts with pharmaceutically acceptable acids.

pharmaceutically acceptable acids.

2. The use according to claim 1, comprising the use of 1,2-naphthoquinone 2-semicarbazone, or its corresponding glucuronidated, 1-(1-hydroxy, 2-naphtyl)-semicarbazide-1-β-O-glucopyranosiduronic acid, with formulae (III) and (IV), respectively,



(III)



(IV)

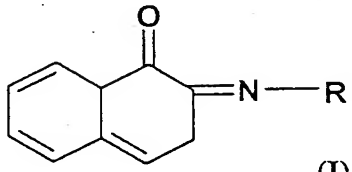
3. The use according to claim 1 or 2, comprising the use of salts formed with mineral acids or organic acids.

4. The use according to any of claims 1 to 3, for making drugs for treating neurological diseases and attacks related to deleterious effects of glutamate released in excess.

5. The use according to claim 4, for making drugs for treating epilepsy, Huntington's disease, amyotrophic lateral sclerosis, spinal amyotrophy, deleterious effects due to the excesses of glutamate released as a result of cerebral accidents of traumatic vascular origin.

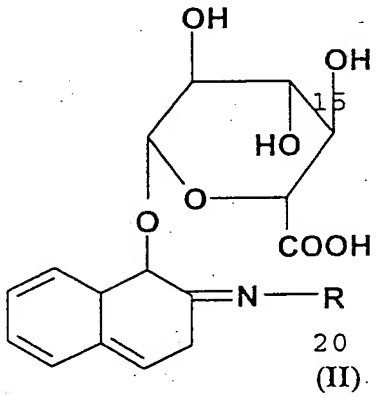
6. The use according to any of claims 1 to 5, characterized in that the drugs are administered orally or via an injectable route.

glutamate by the brain, said derivatives having formula (I)



(I)

wherein R represents a $-NH-CO-NH_2$, $-NH-CO-CH_3$, or -OH group, corresponding glucuronidated derivatives having formula (II)



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as well as addition salts thereof with pharmaceutically acceptable acids.

It is most particularly directed to the use of the 1,2-naphthoquinone 2-semicarbazone, called naftazone according to its international common name, as well as to its corresponding glucuronidated derivative, 1-(1-hydroxy,2-naphthyl)semicarbazide-1-β-O-gluco-pyranosiduronic acid, respectively of formulae, (III) and (IV)

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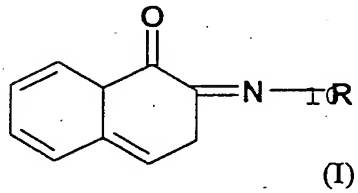
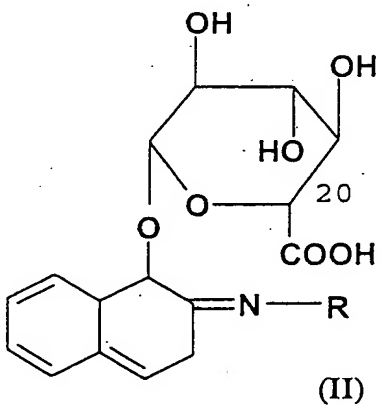
CLAIMS

1. The use of beta-naphthoquinone compounds for making drugs for treating pathologies related to the cytotoxicity of glutamate, these compounds having formula (I)



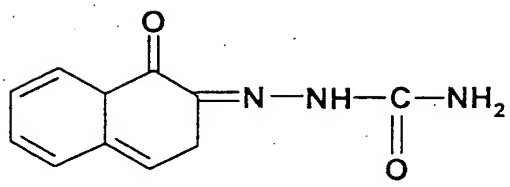
(I)

wherein R represents a -NH-CO-NH_2, -NH-CO-CH_3, or -OH group, glucuronidated derivatives, the corresponding compounds, having formula (II)

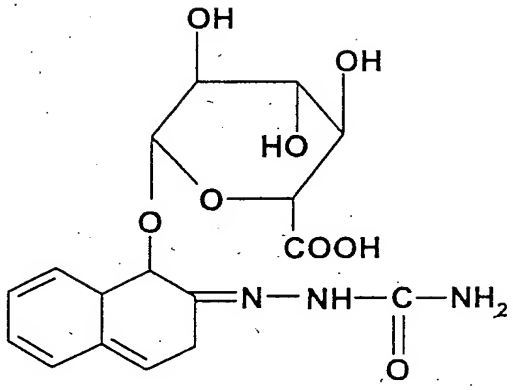


(II)

as well as their addition salts with pharmaceutically acceptable acids.



(III)



(IV)

FIGURE 1

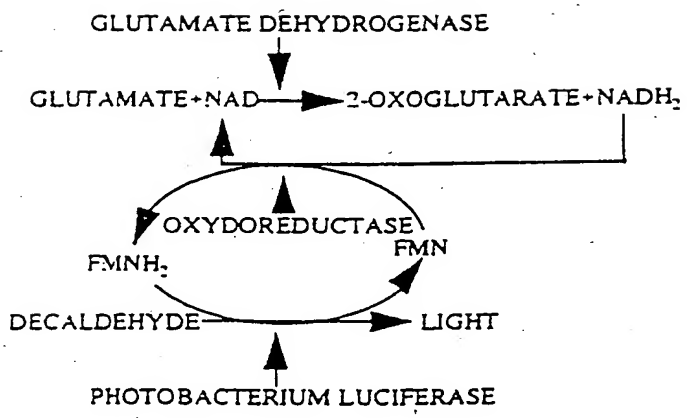

GLUTAMATE DÉHYDROGENASE
GLUTAMATE+NAD
2-OXOGLUTARATE+NADH2
OXYDOREDUCTASE
FMNH2
FMN
DECALDEHYDE
LIGHT
PHOTOBACTERIUM LUCIFERASE

FIGURE 2A

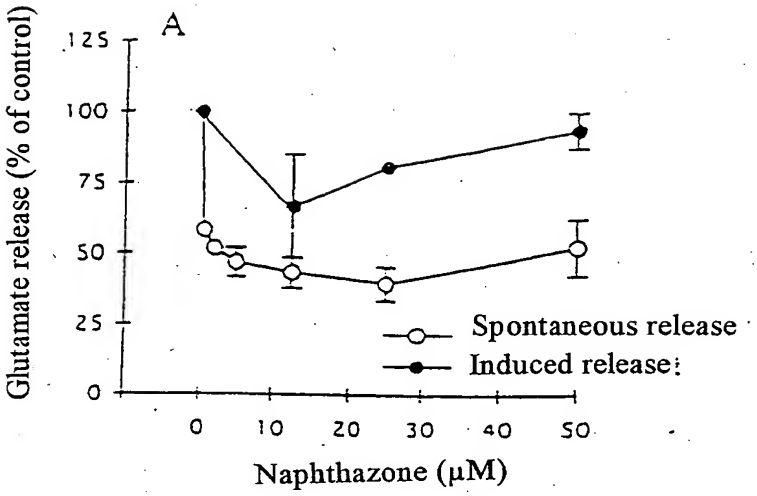


FIGURE 2B